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甘蔗属及其近缘属种的 rbcL 基因序列变异和系统发育初步研究*

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摘要：甘蔗属及其近缘属种在分类关系上非常复杂，存在不少混乱。目前关于它们的分类学研究主要是基于形态学和同工酶水平，而基因水平上的报道很少。我们对甘蔗属及其近缘属种的部分 rbcL 基因片段（1137bp）进行了测序比较，以期探讨 rbcL 基因能否用于研究甘蔗属及其近缘种之间的亲缘关系。序列比较显示 rbcL 基因在甘蔗属和近缘属种间的变异极低，一些来自不同属的个体序列完全一致，而某些同属内不同种个体却略有差异。这些结果表明 rbcL 基因在甘蔗属及其近缘属种之间的进化速率缓慢而不稳定，难以用于系统发育研究。不过，不同聚类方法都将斑茅与其它属种分开，放在玉米与甘蔗属和其它近缘属种分枝的外部，提示斑茅不应列入甘蔗属或蔗茅属，而应独立为一属。

关键词：rbcL；进化；甘蔗属；蔗茅属；斑茅
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Sequence Variation of rbcL Gene and Evolution of *Saccharum* and Related Species

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Abstract：Partial rbcL gene segments（1137bp）were sequenced in species from *Saccharum* and its related species，including one *Arundinaceum* and one *Pennisetum purpureum* sample. Sequence variation of rbcL gene in *Saccharum* and related species were found to be highly conservative，and identical sequences were presented between *Saccharum* and *Miscanthus* ，and *Narenga* and *Miscanthus*，while sequences of some individuals from same genus had some variation. The results suggest that rbcL gene is not a good genetic marker for the phylogenetic study of *Saccharum* and related species. Based on the facts that

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a relatively large sequence divergence exists between *Arundinaceum* and individuals from *Saccharum* or *Erianthus*, *and the similar phylogenetic position of Arundinaceum* in the gene trees that fall out of the clade of" Saccharastrae " that considered, we tentatively suggested that *Arundinaceum* should be in an independent genus rather than being included in *Saccharum* or in *Erianthus*.

Key words: rbcL; Evolution; *Saccharum*; *Erianthus*; *Arundinaceum*

Saccharum L. is a important plant to sugar industry. *Saccharum* mainly includes *S. officinarum* L., *S. sinense* Roxb., *S. barberi* Jeswiet., *S. spontaneum* L. and *S. robustum* Brandes (Chen, 1997). *Saccharum*, *Erianthus* Michx., *Miscanthus* Anderss., *Sclerostachya* (Anderss. ex Hack.) A. Camus and *Narenga* Bor compose " *Saccharum* " Complex. Except *Sclerostachya* (Anderss. ex Hack.) A. Camus., the four other genera are abundant in China. " *Saccharum* " Complex, together with *Imperata* Cyr., *Eccoilopus* Steud., and *Spodiogogon* Trin., form the" Saccharastrae " (Peng, 1990). However, the relationships between *Saccharum* and related species are very controversial (Peng, 1990). Previous studies on the relationships between *Saccharum* and related species were mainly based on morphological and isozyme results (Xiao, 1994; Zheng, 1989), evidence from DNA sequence may express the evolution of *Saccharum* and related species.

S. arundinaceum Retz. has been widely used in breeding of sugarcane. However, its taxonomic status is still an open question. In China, most bontanists classify *Arundinaceum* as a species of *Saccharum*, named as *S. arundinaceum* Retz. While another view, suggesting that *Arundinaceum* should be included in *Erianthus*, named as *E. arundinaceus* (Retz.) Jeswiet., is prevalent in other countries (Peng, 1990). Cai (1984) suggested that it might be better for *Arundinaceum* to be an independent genus than to be included in *Saccharum* or in *Erianthus*. However, Xiao (1994) showed that *Arundinaceum* was similar to *Erianthus* not only in morphological feature, but also in biochemical characters of isozyme and chromosome numbers.

rbcL gene, which locates on plant chloroplast genome, encodes the large subunit of ribulose – 1, 5 – bisphosphate carboxylase (Clegg, 1993). Because of its positional conservation and slow evolutionary rate, which make it easy for alignment and primer designing, rbcL gene has been widely used for phylogenetic analysis of plant at higher taxonomic level (Clegg, 1993; Soltis *et al*, 1990; Hasebe *et al*, 1993; Manen and Natali, 1995; Gaut *et al*, 1997). In current study, we sequenced partial rbcL gene of samples from *Saccharum* and related species, in order to learn whether rbcL gene can be a genetic marker to resolve the relationship among *Saccharum* and related species and to elucidate the systematic position of *Arundinaceum*.

1 Materials and Methods

Fourteen samples from *Saccharum* and related species, including one *Arundinaceum*, and one *Pennisetum purpureum* sample were used in this study (Table 1). They were all from the National Sugarcane Germplasm Bank in Sugarcane Research Institute, Yunnan Academy of Agricultural Sciences.

Table 1　Samples used in the present study, including samples from *Saccharum* and related species, in addition to one *P. purpureum* sample.

Genus	Species
	S. officinarum L.
	S. barberi Jeswiet
	S. sinense Roxb.
Saccharum L.	*S. robustum* Brandes
	S. spontaneum L.
	S. arundinaceum Retz (Endemic species)
Erianthus Michx	*E. rockii* Keng
	E. fulvus Nees
Narenga Bor	*N. fallax* (Balansa) Bor
	N. porphyrocoma (Hance) Bor
Miscanthus Anderss	*M. floridulus* (Labill.) Warb. ex Schum et Laut.
	M. sinensis Anderss.
Imperata Cyr.	*I. cylindrica* L.
Pennisetum	*P. purpureum* Schumach

Genomic DNA was extracted using the methods described by Fang *et al*. (1999). Ten primers were used here for amplification and sequencing: PRL1 (5′ – cgg ggt tcc gcc tga aga agc – 3′), PRL2 (5′ – cgt ttg gag gat cta cga att cc – 3′), PRL3 (5′ – cat tta tgc gct gga gag ac – 3′), PRL4 (5′ – tct cat tat tgc cgc gac aac – 3′), PRL5 (5′ – cgg tgg ctt cag ggg gta tt – 3′), PRH1 (5′ – aag cag cag cta att cag gac tcc – 3′), PRH2 (5′ – ttc ggt cag agc tgg cat atg c – 3′), PRH3 (5′ – tac cta cta ctg tac cgg ag – 3′), PRH4 (5′ – gtc atg cat tac aat agg aac – 3′), and PRH5 (5′ – ccg tac ttg ttc aac tta tcc – 3′). Their relative amplifying locations are shown in Figure 1. Amplification was performed in 50 μL volume following 35 – 40 cycles of 94℃ 30 seconds, 46 – 50℃ 30 seconds, 72℃ 1.5 minutes. PCR products were purified in 1.5% – 2.0% low melting agarose and were sequenced with ABI 377 DNA automatic sequencer. Both strands were sequenced.

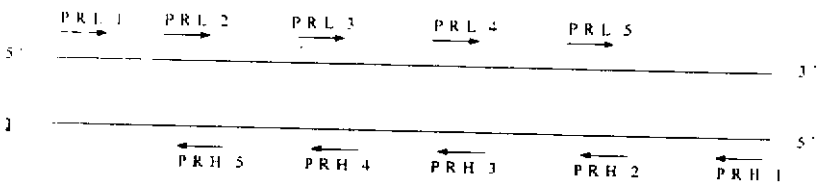

Fig 1. Relative binding locations of ten primers. PRL denotes primers of light chain, PRH denotes primers of heavy chain.

DNA fragments were edited, merged and aligned using DNASTAR software (DNASTAR Inc.). Some published rbcL gene sequences were retrieved from GenBank (see Note of Figure 3) and included here for comparison. After alignment with our sequences and exclusion of ambiguous sites, we constructed phylogenetic trees with unambiguous 1137 bp, using Cypripedium *C. irapeanum* as outgroup. Parsimony analysis was performed with PAUP 3.1.1 (Swofford, 1993). All characters were assigned as unordered and given equal weight. Bootstrap analysis consisted of 1000 heuristic replications. NJ tree was constructed after translation from nucleic acid sequence data to distance data using Kimura 2 – parameter model in MEGA 1.02 (Kumar *et al*, 1993).

2 Results

Partial rbcL gene sequences (1162bp – 1187bp) of samples from *Saccharum* and related species, and one *Pennisetum purpureum* sample were obtained in this study. Some sites in several samples were ambiguous. After exclusion of these ambiguous sites, the results of comparison from the remaining unambiguous 1137 bp showed that rbcL gene sequences in *Saccharum* and ralated species were highly conservative. The sequences from *S. sinense*, *S. robustum*, *M. floridulus*, *S. arundinaceum* and *S. spontaneum* were identical. *S. officinarum* and *S. barberi* were differed from three other species in *Saccharum* by one and two substitutions, respectively. *M. sinensis* and *N. fallax* (Balansa) also shared the same sequence (Figure 2). However, the sequence of *Arundinaceum* Retz. had relatively large discrepancies comparing to those of *Saccharum* and *Erianthus*. Thus it is not suitable to classify *Arundinaceum* either in *Saccharum* or *Erianthus*.

The two gene trees constructed by different methods are similar to each other in the topologies (Figure 3). Both trees could not separate *Saccharum* from other related species in "*Saccharum*" Complex completely. However, *Arundinaceum* did not fall into the clade containing "Saccharastrae" that considered in the trees and *Zea mays*.

3 Discussion

The enzyme ribulose – 1, 5 – bisphosphate carboxylase (Rubisco) is responsible for fixation of carbon dioxide in the Calvin cycle. Rubisco is formed by a 16 – mer structure that includes eight large subunit polypeptides and eight small ones. The encoding gene for the large subunit, rbcL gene, is located in chloroplast genome (Clegg, 1990). Positional conservation of coding information permits the unambiguous alignment of rbcL sequences, which is an important advantage for the study of seed plant evolutionary history. Low evolutionary rate of rbcL gene makes it easy for primer designing, and provides an appropriate marker for the phylogenetic study at higher taxonomic levels (Clegg, 1990). Our results gave supports to the suggestions mentioned above and showed that rbcL gene is not a good genetic marker for phyloge netic study on *Saccharum* and related species by the facts: 1. sequence variations of rbcL gene in *Saccharum* and related species were very small; 2. identical sequences were presented between *Saccharum* and *Miscanthus* and *Narenga* and *Miscanthus*, while some individuals from the same genus had some variation.

Although *S. arundinaceum* has been widely used in sugarcane breeding, its taxonomic status is still controversial. Currently, there are mainly three opinions about the taxonomic position of the species. The arguing points focus on whether *Arundinaceum* should be included in *Saccharum* or in *Erianthus*, or should be an independent genus (Chen, 1997; Peng, 1990; Xiao, 1994; Cai, 1984). Cai (1984) studied the isozyme patterns of peroxidase and esterase in *S. spontaneum*, *S. arundinaceum* and *E. fulvus*, and found that the pattern of *S. spontaneum* was the most complicated, followed by that of *S. arundinaceum*, while the pattern of *E. fulvus* was the simplest. And the features of their nutrient organs also have some differences. Based on these evidences, Cai (1984) suggested that it would be more proper for *Arundinaceum* to be treated as an independent genus than be

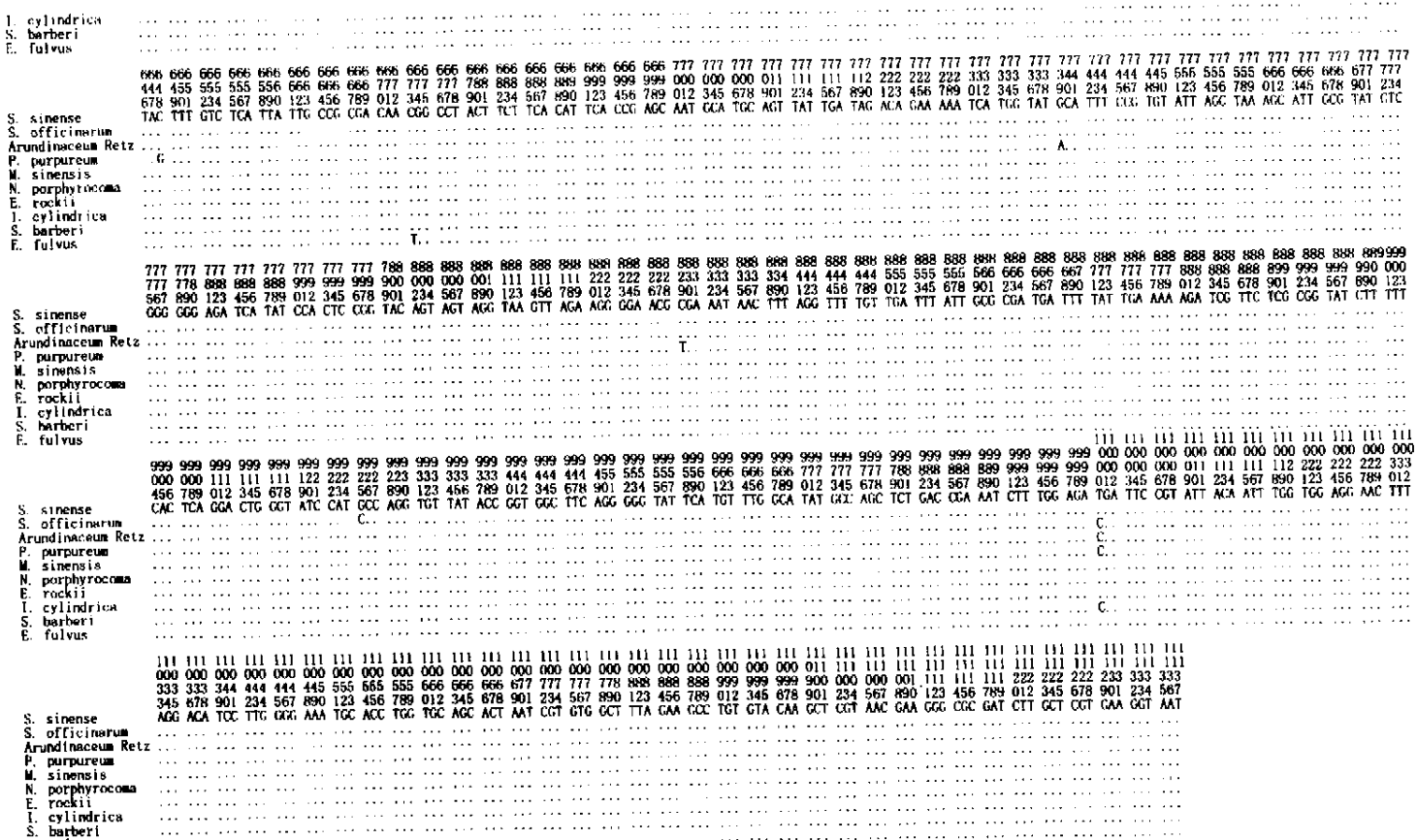

Fig. 2 rbcL sequence compilation of samples studies in the present work. The sequences of *S. sinense* Roxb., *S. bobustum* Brandes., *M. floridulos* (Lahill.) Warb/ex Schum et Laut., Endemic species, and *S. spontaneum* L. were identical. M. sinensis Anderss. And N. fallax (Balansa) Bor were also shared the same sequence.

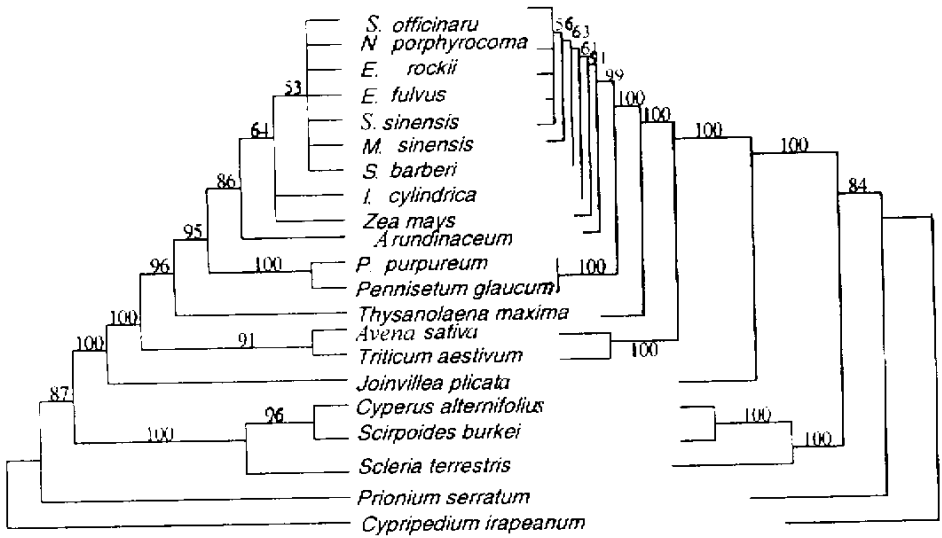

Fig.3 Phylogenetic trees constructed from rbcL sequences. (a) PAUP parsimonious tree; (b) NJ tree. The numbers on the branch denote bootstrap confidence values. Other data retrieved from Genebank for comparison are *Thysanolaena maxima* (U31380), *Avena sativa* (L15300), *Triticum aestivum* (X62117), *Zea mays* (Z11973), *Pennisetum glaucum* (L14623) of grass family (Poaceae); *Scleria terrestris* (Y12947), *Scirpoides burkei* (Y13001), *Cyperus alternifolius* (M91627) in Cyperaceae; *Joinvillea plicata* (L01471) in Joinvilleacaea; *Prionium serratum* (U49223, L11169) in Juncaceae; and *Cypripedium irapeanum* (Z73706) in Orchidaceae.

included in *Saccharum* or in *Erianthus*. Our results support this opinion, for *Arundinaceum* occupied similar phylogenetic position in the two trees, which falling out of the clade that clustered maize (*Z. mays*), *Saccharum* and other related species. Another evidence is that sequence variation between *Arundinaceum* and individuals from *Saccharum* or *Erianthus* are relatively large.

In short, our results showed that rbcL gene was not a proper marker to infer the phylogenetic relationships of *Saccharum* and related species, and *Arundinaceum* would be an independent genus rather than be included in *Saccharum* or in *Erianthus*. Additional evidence from nuclear marker, like ITS sequence, will be necessary to resolve the phylogenetic controversy of *Saccharum* and related species in future studies.

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References:

Cai ZL, 1984. The classification and relationship of sugarcane and its related species [J]. *Sugarcane*, **3**: 15—19

Chen SL，1997. Gramineae（Poaceae）In：Delectis Florae Reipublicae Popularis Sinicae，Agendae Academiae Sinicae edita. Flora Reipublicae Popularis Sinicae，Tomus，10（2）[M]. Beijing：Science Press.

Clegg MT，1993. Chloroplast gene sequences and the study of plant evolution [J]. *Proc Natl Acad Sci USA*，**90**：363—367

Fang YH，Cai Q，Su B，*et al*，1999. Effect of DNA extraction method to RAPD results of 6 Saccharastrae plants [J]. *Journal of Southwest Agriculture*，**12**（1）：1—7

Gaut BS，Clark LG，Wendel JF，*et al*，1997. Comparisons of the molecular evolutionary process at rbcL and ndhF in the grass family（Poaceae）[J]. *Mol Biol Evol*，**14**（7）：769—777

Hasebe M，Ito M，Kofuji R，*et al*，1993. Phylogenetic relationships of ferns deduced from rbcL gene sequence [J]. *J Mol Evol*，**37**：476—482

Kumar S，Tamura K，Nei M，1993. MEGA：molecular evolutionary genetics analysis. Version 1.02 [C]. Pennsylvania：The Pennsylvania State University.

Manen J，Natali A，1995. Comparison of the evolution of ribulose－1，5－biphosphate carboxylase（rbcL）and atpB－rbcL noncoding spacer sequences in a recent plant group，the tribe Rubieae（Rubiaceae）[J]. *J Mol Evol*，**41**：920—927

Peng SG，1990. Sugarcane breeding. Beijing：Agricultural Publishing House，7—62

Soltis DE，Soltis PS，Clegg MT，*et al*，1990. rbcL sequence divergence and phylogenetic relationships in Saxifragaceae sensu lato [J]. *Proc Natl Acad Sci USA*，**87**：4640—4644

Swofford DL，1993. PAUP：phylogenetic analysis using parsimony. Version 3.1.1 [C]. Champaign：Illinois Natural History Survey.

Xiao FH，1994. The comparable studies of isozyme and morphology among *Arundinaceum* Retz. and several species of *Saccharum* and *Erianthus* [J]. *Sugarcane* **1**（1）：22—27

Zheng DS，1989. Isozyme analysis on the relationships between *Saccharum* species and varieties [J]. *Journal of Fujian Agricultural University*，**8**（2）：163—167